

U.S. Patent Application No. 10/088,750
Amendment dated April 22, 2005
Reply to Office Action of January 26, 2005

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 17, line 18, and ending on page 18, line 16, as follows:

The amplification of the mutant fragments was carried out using synthesized DNA primers containing mutation sequences and a high fidelity DNA polymerase (KOD Dash, Toyobo Co., Japan), and the amplified fragments were purified by the agarose electrophoresis, phosphorylated, self-ligated, and transformed in *Escherichia coli*. Substitution of the nucleotide was confirmed by DNA-sequencing. Deletion and substitution of the spiral part of stem loop III was carried out by the fusion PCR (Virology 214, 611-618 (1995)). A forward primer (GGTTAAATTCGAGGTAAAAAATTGCTATA)
(GGTTAAATTCAGGTAAAAAATTGCTATA), SEQ ID NO. 8, containing nt6029-6050 and nt6062-6080 of PSIV sequence and a reverse primer containing nt27-5 of pT7Blue (Novagen, Inc.) were synthesized for the initial amplification to delete nucleotide (nt) 6051-6061. Then, a reverse primer (CCTCGAAATTAAACCAGATCACATAGTCAGCTTC), SEQ. ID. NO. 9, containing nt6043-6029 and nt6017-5998 of PSIV sequence and a forward primer containing nt28-47 of pT7Blue (Novagen, Inc.) were synthesized for another initial amplification to delete nt6028-6018. Underlines of these primers indicate 15nt overlap of the fusion PCR. After each initial amplification using these two primer sets, two amplified DNA fragments were mixed and fused according to the description in Ref. 17. The final amplification was carried out using primers carrying nt28-47 and nt27-5 of pT7Blue, and the amplified DNA fragments were

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purified with a gel, phosphorylated, and ligated. The initial amplification was carried out using longer primers containing a substituted nucleotide to substitute the helical part.